Rac1 regulates peptidoglycan-induced nuclear factor-kB activation and cyclooxygenase-2 expression in RAW264.7 macrophages by activating the phosphatidylinositol 3-kinase/Akt pathway

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Abstract

Previously, we found that peptidoglycan (PGN), a cell wall component of the gram-positive bacterium Staphylococcus aureus, may activate the Ras/Raf-1/extracellular signal-regulated kinase (ERK) pathway, which in turn initiates IkappaB kinases alpha/beta (IKKalpha/beta) and nuclear factor-kappaB (NF-kappaB) activation, and ultimately induces cyclooxygenase-2 (COX-2) expression in RAW 264.7 macrophages. In this study, we further investigated the roles of Rac1, phosphatidylinositol 3-kinase (PI3K), and Akt in PGN-induced NF-kappaB activation and COX-2 expression in RAW 264.7 macrophages. PGN-induced COX-2 expression was attenuated by a Rac1 dominant negative mutant (RacN17), PI3K inhibitors (wortmannin and LY 294002), and an Akt inhibitor (1L-6-hydroxymethyl-chiro-inositol2-[(R)-2-O-methyl-3-O-octadecylcarbonate]). PGN-induced PGE(2) release was also inhibited by RacN17. Treatment of RAW 264.7 macrophages with PGN caused the activation of Rac and Akt. PGN-induced Akt activation was inhibited by RacN17, LY 294002, and the Akt inhibitor. Stimulation of RAW 264.7 macrophages with PGN resulted in an increase in IKKalpha/beta phosphorylation and p65 Ser536 phosphorylation; these effects were inhibited by RacN17, LY 294002, an Akt inhibitor, and an Akt dominant negative mutant (AktDN). The PGN-induced increases in kappaB-luciferase activity were also inhibited by RacN17, wortmannin, LY 294002, an Akt inhibitor, and AktDN. Treatment of macrophages with PGN induced the recruitment of p85alpha and Rac1 to Toll-like receptor 2 (TLR2) in a time-dependent manner. These results indicate that PGN may activate the Rac1/PI3K/Akt pathway through the recruitment of p85alpha and Rac1 to TLR2 to mediate IKKalpha/beta activation and p65 phosphorylation, which in turn induces NF-kappaB transactivation, and ultimately causes COX-2 expression in RAW 264.7 macrophages.